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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Antipsychotic drugs (APDs) are widely used psychotropic medications, though they have significant metabolic side effects. While the mechanisms for these metabolic disturbances are poorly understood, the single known unifying property of all APDs is their blockade of the dopamine D_2 (D2R) and D_3 (D3R) receptors. We therefore hypothesize that D2R and/or D3R mediate the metabolic side effects of APDs both centrally in the hypothalamus and peripherally in pancreas, areas critical for metabolic regulation. In Year 1 of this award, we have completed the design of a D3R-flox mouse in order to selectively knock out expression of D3R in the hypothalamus and pancreatic beta cells. The resulting transgenic mice are being tested to confirm the successful production of the strain. In parallel, we have completed construction of novel inducible transgenic hypothalamic- and pancreatic beta cell-specific D2R knockout (KO) mice. Additionally, using pancreatic islets isolated from beta cell-selective D2R KO mice and complete D3R KO mice, we found diminished inhibition of stimulated insulin secretion in both strains relative to littermate controls, suggesting a role for both receptors in mediating insulin secretion.

15. SUBJECT TERMS
None listed

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1. INTRODUCTION

Antipsychotic drugs (APDs) are widely used psychotropic medications for numerous psychiatric illnesses including schizophrenia, posttraumatic stress disorder and depression. However, these medications also have significant metabolic side effects characterized by substantial weight gain, glucose intolerance, insulin resistance, hypertension and dyslipidemia as well as increased risks for type 2 diabetes and cardiovascular disease. Indeed, the prevalence of these APD-induced metabolic side effects in Veterans is more than twice that of the general population. However, the mechanisms for these metabolic disturbances are not well understood. Significantly, all APDs cause these side effects to differing degrees and ultimately result in life-shortening morbidity. A potentially important clue is that the single known unifying property of all APDs is their blockade of the dopamine D_2 (D2R) and D_3 (D3R) receptors, suggesting a role for these receptors in APD metabolic side effects. Consistent with this, D2R and D3R are expressed both centrally in the hypothalamus in regions mediating appetite and feeding behavior as well as peripherally in insulin-releasing pancreatic beta cells, key regulators of metabolism. We previously showed that activation of pancreatic beta cell D2R and D3R inhibited glucose-stimulated insulin secretion (GSIS) and that APD-induced receptor inhibition disrupted this regulatory mechanism. Thus, our central hypothesis is that D2R and/or D3R are critical regulators of metabolism and mediate the metabolic side effects of APDs both centrally in the hypothalamus and peripherally in pancreas. However, the relative contributions of peripheral and central D2R and D3R to APD-induced metabolic dysregulation are unknown. To disentangle these mechanisms, in partnership with PI Dr. Zachary Freyberg, we will aim to do the following: (1) to identify contributions of hypothalamic D2R and D3R action in APD-induced weight gain and metabolic dysregulation *in vivo*; (2) to identify the relationship of peripheral D2R and D3R to APD-induced weight gain and metabolic dysfunction *in vivo*; and (3) to identify APD-mediated effects on insulin and DA release in pancreatic beta cells using real-time imaging. Key to these aims is the generation of tissue-specific D2R and D3R knockout (KO) mice targeting either hypothalamus or pancreatic beta cells. In the short term, our work will elucidate the anatomical and functional mechanisms of APD-induced metabolic side effects. In the longer term, we will use our findings to develop better-targeted APDs that can selectively reverse these drugs' metabolic side effects while preserving their clinical efficacy.

2. KEYWORDS

Keywords relevant to the work proposed here include:

1. Antipsychotic drug (APD)
2. Dopamine (DA)
3. Dopamine D_2 Receptor (D2R)
4. Dopamine D_3 Receptor (D3R)
5. Insulin
6. Glucose-stimulated insulin secretion (GSIS)
7. Diabetes
8. Metabolism

3. ACCOMPLISHMENTS

- **What were the major goals of the project?**

The major goals of the project as stated in the approved SOW are as follows:

A. Metabolic characterization of hypothalamus-specific D2R and D3R knockout mice in the presence or absence of APD treatment

B. Metabolic characterization of pancreatic beta cell-specific D2R and D3R knockout mice in the presence or absence of APD treatment

C. Treatment with domperidone to determine whether peripheral D2R/D3R blockade alone can produce relevant metabolic disease

D. Determine the precise contributions of D2R and D3R to glucose-stimulated insulin and dopamine release using pancreatic islets from pancreatic beta cell-selective D2R and D3R knockout mice as well as wildtype controls

E. Determine effects of APDs on kinetics of real-time glucose-stimulated insulin and dopamine release in wildtype and beta cell-specific D2R or D3R knockout mouse pancreatic islets.

- **What was accomplished under these goals?**

In the course of the reporting period for Year 2 of this award, we conducted studies to address each of the major goals of the project as follows:

I. Metabolic characterization of hypothalamus-specific D2R and D3R knockout mice in the presence or absence of APD treatment

To identify the likelihood that central versus peripheral D2 receptors are implicated in APD-mediated metabolic dysfunction, we began by evaluating the effects of chronic daily intraperitoneal administration of the D2R agonist bromocriptine to animals maintained chronically on high carbohydrate/ high fat diet that we have shown under this grant to reliably drive glucose and insulin in tolerance in male and female C57/B6J mice. We found in a cohort of 8 males and 8 females that such chronic administration markedly improves oral glucose and intraperitoneal insulin tolerance, and tends to increase oxygen consumption, energy expenditure, locomotor activity, and respiratory quotient, but without significantly affecting adiposity. These data suggest a primary benefit of D2R stimulation on glucose metabolism and or insulin sensitivity independently of adiposity, in models of glucose and insulin intolerance characteristic of type 2 diabetes. We subsequently found in separate cohorts of 8 male and 8 female C57/B6J mice receiving equivalent single daily intraperitoneal dosed injections of the peripherally selective D2R agonist AB-01 (a methiodide derivative of bromocriptine provided by Dr. Amy Newman at NIDA), failed to drive improvements in any of the metabolic variables characterized, including glucose and insulin tolerance. These data distinctly point, for the first time, to a significant role for central and not peripheral D2 receptors in modulating glucose and insulin tolerance in prediabetes, and strongly support our subsequent planned analyses employing central vs. peripheral D2 receptor ablation in APD-induced metabolic disease.

We now have available existing D2R-flox corssed with Nkx2.1-cre hypothalamic expression mice. This series of breeding steps permitted us to generate the final Nkx2.1-cre hemizygous/D2R-flox homozygous mice.

In order to characterize the metabolic consequences of hypothalamus-specific knockout of D3R, we have now confirmed successful generation of the D3 flox strain, accordingly we will receive shipment of these animals to Einstein quarantine facility and will begin breeding them to Nkx2.1-cre mice which selectively express the Cre recombinase throughout the hypothalamus. The resulting Nkx2.1-cre hemizygous/D3R-flox homozygous mice will have selective knockout of D3R across the hypothalamus.

Significantly, we have successfully secured complete renewes IRB approval for all of our animal work during this reporting period.

II. Metabolic characterization of pancreatic beta cell-specific D2R and D3R knockout mice in the presence or absence of APD treatment

- As of September 2017, we have completed construction of a transgenic mouse strain with transgenes for inducible D2R knockout specifically in pancreatic beta cells. In the course of designing the breeding strategy, we were able to use a new pancreatic beta cell-specific expression driver, Mip1-cre/ERT which has two important advantages

over previous beta cell cre driver strains: (1) it is more specific to beta cells with virtually no off-target expression in other organ systems including brain; and (2) it is inducible, allowing us to avoid possible developmental effects that could confound our interpretation of the results. Following approximately 12 months of crosses, we have now successfully generated the final desired genotype of hemizygous cre; homozygous D2R-flox mice required to completely knockout expression of D2R selectively in pancreatic beta cells. With this transgenic mouse strain established, they can now be amplified in numbers sufficiently powered to resolve potential effects of D2R absence on APDs' effects on glucose tolerance, insulin resistance, glucose stimulated insulin secretion, adiposity and weight gain.

- The Mip1-cre/ERT mice are also being prepared for crosses to the new D3R-flox mouse strain in order to begin construction of an inducible beta cell-specific D3R knockout mouse line. We expect the crosses to begin in December 2017.

- We have performed chronic intravenous and intra-arterial catheterization in these animals in preparation for insulin clamp studies of hepatic insulin action; we have found that our diet induced obese, glucose and insulin intolerant animals survive the surgery well, and have stable elevated basal glucose levels, and are thus amenable to the planned clamp procedures well as the clamp level for both hyperinsulinemic- euglycemic and euinsulinemic- hyperglycemic clamps.

- **What opportunities for training and professional development has the project provided?**

Nothing to Report.

- **How were the results disseminated to communities of interest?**

Nothing to report. As results proceed we will plan to submit an abstract to the American Diabetes Association annual meeting.

What do you plan to do during the next reporting period to accomplish the goals?

I. Metabolic characterization of hypothalamus-specific D2R and D3R knockout mice in the presence or absence of APD treatment

- In the next reporting period, we will conduct weekly measurement of weights and food consumption in hypothalamus-specific D2R (and wildtype littermate controls) treated with either with first-generation APD haloperidol or second-generation APD olanzapine (via i.p. administration). We will also measure serum fasting glucose and insulin levels in hypothalamus-specific D2R knockout mice and wildtype littermate control mice in the presence or absence of APD treatment; serum will be collected at weeks 13 and 26 of APD treatment.

- With the completion of construction of the D2/D3R-flox mice, we will also begin crosses to establish hypothalamus-specific D3R knockout mice (Nkx2.1-cre hemizygous, D2/D3R-flox homozygous mice). This process is expected to take approximately 10-12 months.

- As a follow- up study to our bromocriptine work, we will provide chronic injections of 10-50 x lower does of bromocriptine directly into the lateral and third cerebral ventricles to identify the degree to which selective central and not peripheral stimulation of D2 receptors are sufficient drive improvements in glucose and insulin tolerance seen following chronic peripheral administration. We can then employ our inducible hypothalamic-selective D2 knockout mice to identify the degree to which hypothalamic D2 receptors can improve systemic glucose homoestasis.

II. Metabolic characterization of pancreatic beta cell-specific D2R and D3R knockout mice in the presence or

absence of APD treatment

- We will first characterize the quantity and duration of tamoxifen necessary to induce successful deletion of D2R in our inducible pancreatic beta cell-specific and Nkx2.1 hypothamic neuron specific D2R knockout mice. Once we have confirmed deletion of D2R in pancreatic islets and hypothalamic neurons, we will begin characterizing the metabolic status of these animals from week 3 of life onwards following completion of weaning. Specifically, we will conduct weekly measurement of weights and food consumption in beta cell-specific D2R (and wildtype littermate controls) treated with either with first-generation APD haloperidol or second-generation APD olanzapine (via i.p. administration). We will also measure serum fasting glucose and insulin levels in hypothalamus-specific D2R knockout mice and wildtype littermate control mice in the presence or absence of APD treatment; serum will be collected at weeks 13 and 26 of APD treatment.
- In parallel with generation of beta cell-specific D2R knockout mice, we will also begin crosses to establish inducible pancreatic beta cell-specific D3R knockout mice (Mip1-cre/ERT hemizygous, D3R-flox homozygous mice). This process is expected to take approximately 10-12 months.

III. Treatment with domperidone to determine whether peripheral D2R/D3R blockade alone can produce relevant metabolic disease

- We will use our high fat high carbohydrate diet to compare effects of domperidone on the rate of development of insulin resistance both in wildtype as well as in Nkx2.1 hypothalamic neuronal and beta cell-specific D2/D3 double knockout mice. Besides insulin resistance, we will look at other markers of metabolic disease including adiposity, fatty liver and pancreatic beta cell mass.

- As each of these three above aims proceeds with the new strains available, we will perform insulin clamp studies to assess the efficacy of hepatic insulin action as well.

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?**

In the short term, the impact of our present studies points strongly to a novel central role for D2R in improving glucose and insulin tolerance, and facilitate our ability to experimentally differentiate pancreatic beta cell vs hypothalamic neuronal D2 contributions to system insulin sensitivity and glucose tolerance in well-characterized rodent models of pre-diabetes.

- **What was the impact on other disciplines?**

In the longer term, the knowledge resulting from our work may directly lead to development of better APDs free of metabolic side effects. This could significantly reduce serious morbidity and mortality from medication-associated type II diabetes and cardiovascular disease. Moreover, better understanding the mechanisms by which dopamine and dopamine receptors mediate insulin release may also significantly contribute to our fundamental understanding of obesity and lead to novel treatments. Since APD-induced metabolic disturbances also increase risks of developing type II diabetes and Alzheimer's disease, further elucidating the mechanisms of APD-induced weight gain may also lead to fundamental insights into the mechanisms for development of these disorders.

- **What was the impact on technology transfer?**

Nothing to Report.

- **What was the impact on society beyond science and technology?**

Nothing to Report.

5. CHANGES/PROBLEMS

Nothing to Report.

6. PRODUCTS

- **Publications, conference papers, and presentations**

Journal publications
No peer-reviewed articles or papers appeared in scientific, technical or professional journals.

Books or other non-periodical, one-time publications
Nothing to report.

Other publications, conference papers, and presentations
Nothing to report

- **Website(s) or other Internet site(s)** Nothing to Report.

- **Technologies or techniques** Nothing to Report.

- **Inventions, patent applications, and/or licenses** Nothing to Report.

- **Other Products** Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

• Name:	Gary J. Schwartz, Ph.D.
• Project Role:	Principal Investigator
• Researcher Identifier (*e.g.* ORCID ID):	ORCID ID: 0000-0003-0446-5553
• Nearest person month worked:	4
• Contribution to Project:	Dr. Schwartz has designed and analyzed all experimental data in the areas of metabolic phenotyping, including body weight, adiposity, glucose tolerance, insulin tolerance, and preliminary food intake and calorimetric assessments of energy expenditure.
• Funding Support:	National Institutes of Health/R01 NIDDK

• Name:	Licheng Wu
• Project Role:	Technician D
• Researcher Identifier (*e.g.* ORCID ID):	N/A
• Nearest person month worked:	12
• Contribution to Project:	Mr. Wu has performed dietary testing, glucose and insulin injections and gavage, animal maintenance and genotyping, blood sampling for glucose and insulin tolerance tests, vascular surgical procedures for clamps

	and initial metabolic phenotyping.
• Funding Support:	N/A

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** Nothing to Report.

- **What other organizations were involved as partners?**

Nothing to Report

8. <u>SPECIAL REPORTING REQUIREMENTS</u>

- **<u>Collaborative Awards</u>**

We have worked closely with the Initiating PI of this award, Dr. Zachary Freyberg. Dr. Freyberg has submitted a separate report independently of the Partnering PI that summarizes his progress over the course of the last reporting period (10/1/2016-09/30/2017).

9. <u>APPENDICES</u>

<u>None.</u>